IN THE SPECIFICATION:

At page 25, please replace the paragraph beginning on line 13 with the following amended paragraph.

In addition, the primers designed may be compared to the known sequences in the template nucleic acid, to avoid non specific hybridization of the primers to the template nucleic acid. For example, primers for use in detecting nucleotides in human genomic DNA can be "blasted" against human GenBank sequences, e.g., at the National Center for Biotechnology Information (NCBI) at ~~http://~~www.ncbi.nlm.nih.gov/.

At page 29, please replace the paragraph beginning on line 13 with the following amended paragraph.

The nucleotides are preferably added to a final concentration from about 0.01 ~~uM~~ μM to about 100 ~~uM~~ μM, and preferably about 0.1 ~~UM~~ μM to 10 ~~UM~~ μM in the reaction. The concentration of ligase to add is described in the following section. Preferred amounts of Taq DNA Polymerase Stoffel fragment include 0.05 ~~u/ul~~ U/μL. A typical reaction volume is about 10 to 20 ~~ul~~ μL. Preferred amounts of template and probe DNA are also described in the following section.

Please replace the paragraph beginning on page 29, line 30 and ending on page 30, line 8, with the following amended paragraph.

Background signals may also result from the presence of the "correct" nucleotide in the reaction due to the presence of nucleotides in reagents, and its attachment to the probe. Contamination of reagents with nucleotides can be reduced by treatment of the reagents with an enzyme that degrades free nucleotides. Preferred enzymes include Apyrase and

~~phosphotases~~ phosphatases, with the former being especially preferred. As described in the Examples, Apyrase is usually added to the reaction prior to the addition of the one or more dNTPs, at about a concentration of 0.5 ~~mU/ul~~ mU/ μL in a typical reaction of about 20~~ul~~ μL. Generally, the reactions are then incubated at 20°C for a few minutes to up to 30 minutes. The enzyme is then denatured by incubation of the reaction for about 5 to 10 minutes at 95°C. Alternatively alkaline phosphatases may be used such as, e.g. shrimp alkaline phosphatase.

At page 30, please replace the paragraph beginning on line 25 with the following amended paragraph.

The conditions for carrying out the ligation will depend on the particular ligase used and will generally follow the manufacturer's recommendations. For example, preferred Ampligase concentrations are from about 0.0001 to about 0.001 ~~u/ul~~ U/μL, and preferably about 0.0005 ~~u/ul~~ U/μL. Preferred concentrations of probe nucleic acids are from about 0.001 to about 0.01 ~~picomoles/ul~~ picomoles/ μL and even more preferably, about 0.015 ~~picomoles/ul~~ picomoles/ μL. Preferred concentrations of template nucleic acids include from about 1 ~~zeptomole/ul~~ zeptomole/μL to about 1 ~~attomole/ul~~ attomole/μL, most preferably about 5 ~~zeptomoles/ul~~ zeptomoles/μL. A typical reaction is performed in a total of about 20 ~~ul~~ μL.

At page 58, please replace the paragraph beginning on line 17 with the following amended paragraph.

Eight reactions were conducted in parallel in which one of two template DNAs, differing from each other by a single nucleotide (referred to herein as "SNP"), were incubated with or without one of two oligonucleotide probes. The different combinations are set forth in Table 1. The template DNA S7 is 600 bp long double stranded DNA amplified from S. cerevisiae strain S288C, which includes the nucleotide sequence 5' ATCTCGGGATATCAGACTTAGCGGCACCGTCCTCACCG 3' (SEQ

ID NO:[[10]] 1): 1 and template DNA Y7 is 600 bp long double stranded DNA from S. cerevisiae strain YJM789, which includes the nucleotide sequence 5' ATCTCGGGATATCAGACTTAGCGGTACCGTCCTCACCG 3'(SEQ ID NO:[[11]]2). The two template DNAs are identical except in the underlined nucleotide. The oligonucleotide probe "S" (also referred to as Y2:L: S288C) has the nucleotide sequence 5'CCGCTAAGTCTGATATCCCGAGAT/GTCCACGAGGTCTCTAGTC/GACCTGC AGCGTACG/CGGACCTCAAGTGAAGTACA/CGGTGAGGACGGT/G 3' (SEQ ID NO:[[12]]3); and the oligonucleotide probe "Y" (also referred to as Y2:L: yjm789) has the nucleotide sequence 5'CCGCTAAGTCTGATATCCCGAGAT/GTCCACGAGGTCTCTAGTC/GACCTGC AGCGTACG/CGGACCTCAAGTGAAGTACA/CGGTGAGGACGGT/A 3' (SEQ ID NO:[[13]]4). The "/" in the probe sequences indicate the different parts of the probe: homology 1/primer 1/primer 2/barcode/homology 2/SNP. The oligonucleotide probe Y is identical to probe S, except that the 3' most base is complementary to the SNP nucleotide in template DNA Y7.

At page 59, please replace the paragraph beginning on line 7 with the following amended paragraph.

A ligase mix was prepared by combining (per reaction): 8 ~~ul~~ µL of 5x Tth ligase buffer (from Marsh Biomedical, Rochester, New York); 0.32 ~~ul~~ µL of Tth ligase (from Marsh Biomedical, Rochester, New York) and 29.7 ~~ul~~ µL of water. To the 38 ~~ul~~ µL of ligase mix, 1 ~~ul~~ µL of template DNA at 10 ~~pmol/ul~~ pmol/µL was added. The reaction was incubated for 60 minutes at 55°C to hybridize the template DNA and the probe and to ligate the 3' and 5' ends of the oligonucleotide probe. To 12.5 ~~ul~~ µL of this reaction was then added 37.5 ~~ul~~ µL of PCR mix, prepared by mixing (per reaction) 5 ~~ul~~ µL of 10 x Taq Gold buffer (from PE Biosystems, Foster City, CA) ; 6 ~~ul~~ µL dNTPs at 1.25 mM; 0.2 ~~ul~~ µL of AmpliTaq Gold DNA Polymerase at 5 ~~u/ul~~ u/µL (from PE Biosystems,

Foster City, CA) 1 ~~ul~~ μL of primer p1BAR at 10 ~~pmol/ul~~ pmol/ μL; 1 ~~ul~~ μL of primer P2 at 10 ~~pmol/ul~~ pmol/ μL; and 24.3 ~~ul~~ μL of water. The primer p1Bar has the nucleotide sequence 5' GACTAGAGACCTCGTGGAC 3' (SEQ ID NO:[[1]]5) and the primer P2 has the nucleotide sequence 5' GACCTGCAGCGTACG 3' (SEQ ID NO:[[2]]6). The reactions were then incubated for 10 minutes at 95°C to denature the template DNA, followed by 14 cycles of 95°C for 20 seconds; 57°C (decreasing by 0.5 degrees each cycle) for 1 minute; followed by 16 cycles of 95°C for 20 seconds; 50°C for 45 seconds; followed by incubation at 4°C.

At page 59, please replace the paragraph beginning on line 21 with the following amended paragraph.

20 ~~ul~~ μL of each of the amplification products were then subjected to electrophoresis on a 2% weight/volume agarose gel , and the amplification products were visualized by ethidium bromide staining and U.V. light. The results indicate the presence of a band of about 100 nucleotides in the lanes containing the reaction products in which the probe contains the complementary SNP nucleotide to that present in the template DNA, but not in the other lanes. Thus, probe S identifies the SNP on the template DNA S7 and probe Y identifies the SNP on the template DNA Y7. No product is amplified from a reaction mixture containing template DNA S7 and probe Y or template DNA Y7 and probe S.

At page 60, please replce the paragraph beginning at line 10 with the following amended paragraph:

Template DNA Y1:TOS:T:
5' ACATTTAGATCTGCAGTTTCTAATATGAATTCAGTGGAAAAT 3' ~~(SEQ ID NO: 14)~~ (SEQ ID NO:7);
Template DNA Y2:TO S:C:

5' TCGGGATATCAGACTTAGCGGCACCGTCCTCACCGT 3' ~~(SEQ ID NO: 15)~~ (SEQ ID NO:8);

Template DNA Y3:TO S:A:

5' GATCAAATGCGACCATATTCATCAAACTTATAGGCG 3' ~~(SEQ ID NO:16)~~ (SEQ ID NO:9);

Template DNA Y5:TO S:G:

5' CCAGTCCCTTGAGTTCGCGAATAGTAATTTTGGTGATACCTG 3' ~~(SEQ ID NO: 17)~~ (SEQ ID NO:10);

Probe Y1:PL:119:31 (also referred to as SNP1):

5'GAAACTGCAGATCTAAATGTACC/UGTCCACGAGGTCTCTAGTC/TGTAAA ACGACGGCCAGTU/GCTGGAGTTCGCACGCTATA/ATTTTCCACTGAATTCAT ATT 3' ~~(SEQ ID NO: 18)~~ (SEQ ID NO:11);

Probe Y2:PL:C:119:55 (also referred to as SNP2):

5'CCGCTAAGTCTGATATCCCGAGAT/UGTCCACGAGGTCTCTAGTC/TGTAAA ACGACGGCCAGTU/CAAAGGTGGAGCTGCACACT/TTTAAA/ACGGTGAGGA CGGT 3' ~~(SEQ ID NO: 19)~~ (SEQ ID NO:12);

Probe Y3:PL:C:119:131(also referred to as SNP3):

5'ATGGTCGCATTTGATCGAG/UGTCCACGAGGTCTCTAGTC/TGTAAAACGA CGGCCAGTU/GCCTGGGTTACGTGTCTACT/TTTAAA/CGCCTATAAGTTTGAT GAA 3' ~~(SEQ ID NO: 20)~~ (SEQ ID NO:13); and

Probe Y5:PL:119:167(also referred to as SNP5):

5'GCGAACTCAAGGGACTGGTAC/UGTCCACGAGGTCTCTAGTC/TGTAAAAC GACGGCCAGTU/GCAATATGTAACTCTCTGGG/CAGGTATCACCAAAATTACT ATT 3' ~~(SEQ ID NO: 21)~~ (SEQ ID NO:14).

At page 61, please replace the paragraph beginning on line 11 with the following amended paragraph.

A DNA mix was prepared by mixing (per reaction) 2 ~~ul~~ μL of pfu ligase buffer (from

Stratagene, San Diego, CA); 0.1 ~~mul~~ μL of template oliogonucleotide at 400 ~~fmoles/ul~~ fmoles/μL; 0.4 ~~ul~~ μL of probe oligo (also referred to as "barcode oligo") at 10 ~~pmoles/ul~~ pmoles/μL; and 17.5 ~~ul~~ μL of water. The DNA was denatured by incubating these reactions at 95°C for 5 minutes. The nucleic acids were then annealed by incubating the reactions at 65°C for one hour. The final template amount was 40 femtomoles/reaction, and that of the probe oligonucleotide was 4 picomoles/reaction. To each reaction, 20 ~~ul~~ μL of prewarmed (1 minute at 65°C) polymerase/ligase/dNTP mix was added. This mix was prepared by combining (per reaction) 2 ~~ul.~~ μL of 10 x pfu ligase buffer (from Stratagene, San Diego, CA); 2 ~~ul~~ μl of one dNTP at 1 mM; 0.05 ~~ul.~~ μL of Taq DNA Polymerase Stoffel fragment (from PE Biosystems, Foster City, CA) at 10 ~~u/ul~~ U/μL; 1 ~~ul~~ μL of pfu Ligase (from, Stratagene, San Diego, CA) at 4 ~~u/ul~~ U/μL; and 14.95 ~~ul~~ μL of water. The 40 ~~ul~~ μL reactions were incubated at 65°C for 10 minutes.

At page 61, please replace the paragraph beginning on line 22 with the following amended paragraph.

The template DNA was then subjected to rolling circle amplification as follows. 4 ~~ul~~ μL of the above reactions was added to 32 ~~ul~~ μl of RCA mix prewarmed at 65 °C for 10 minutes. RCA mix was prepared by combining (per reaction) 4 ~~ul~~ μL of 10x Vent buffer (from New England Biolabs, Beverly, MA); 2 ~~ul~~ μL of DMSO; 6.4 ~~ul~~ μlof Vent DNA pol. Exo- at 2 ~~u/ul~~ U/μL (NEB); 0.36 ~~ul~~ μL of RCA primer at 100 ~~pmole/ul~~ pmole/μL; 0.93 ul of T4 gene 32 Protein at 1.7 mg/ml (USB); 0.4 ml of MgS04 at 100 mM; and 17.91 ~~ul~~ μL of water. The nucleotide sequence of the RCA primer contains at its 5' end the complement of a portion of the sequence of primer 2, followed by the sequence of primer 1 and has the nucleotide sequence 5' GTCGTTTTACAGACTAGAGACCTCGTGGAC 3' (SEQ ID NO:[[22]]15). The reactions were then incubated at 92 °C for 3 minutes (heat denaturation), following which, 4 ~~ul~~ μL of prewarmed dNTP mix containing 4 mM of all four nucleotides was added, and the reactions were further incubated at 65.5 °C for 4.5 hours. This

amplification results in the synthesis of a long strand having at its 5' end the RCA primer, followed by the rest of primer 2-primer 1-HR1-HR2-tag-primer 2-[primer 1-HR1-HR2-tag-primer 2-]n.

At page 62, please replace the paragraph beginning on line 3 with the following amended paragraph.

For the PCR amplification step, two reactions were done for each of the template/probe combinations by combining 1 ~~ul~~ μL of each of the above reactions with 19 ~~ul~~ μL of PCR mix containing (per reaction) 2 ~~ul~~ μL of 10 x Taq Gold buffer (from PE Biosystems, Foster City,CA); 0.75 ~~ul~~ μL of dNTPs at 4.0 mM; 0.15 ~~ul~~ μL of AmpliTaq gold DNA Polymerase at 5 ~~u/ul~~ U/μL (PE); 0.16 ~~ul~~ μL of P1bar primer (SEQ ID NO:[[1]]5) at 100 ~~pmol/ul~~ pmol/μL; 0.16 ~~ul~~ μL of M13 primer (i.e., primer 2) at 100 ~~pmol/ul~~ pmol/μL; 2 ul of ~~MgCl2~~ $MgCl_2$ at 25 mM; and 13.78 ~~ul~~ μL of water. The nucleotide sequence of the M13 primer is 5' TGTAAAACGACGGCCAGT 3' (SEQ ID NO:[[3]]16). The PCR reactions were denatured for 5 minutes at 95 °C and then subjected to either 15 or 25 cycles of 20 seconds at 95 °C and 1 minute at 50 °C.

At page 62, please replace the paragraph beginning on line 11 with the following amended paragraph.

20 ~~ul~~ μL of each of the reactions were then subjected to gel electrophoresis in 2% agarose, and the products visualized as described in Example 1. The results indicate that in one of each of the four reactions containing a different dNTP each, amplification product is obtained with the dNTP that is complementary to the SNP in the DNA. For example, more amplification product was detected in the reaction in which dATP was added to the probe containing a thymidine as SNP nucleotide, compared to the reactions in which dCTP, dGTP or dTTP was added.

At page 62, please replace the paragraph beginning at line 28 with the following amended paragraph:

The template DNAs and probes were identical to those used in Example 1: The two template DNAs used were the 600 bp amplicons designated S7 and Y7, comprising ~~SEQ ID NO: 10 and 11~~ (SEQ ID NOS:1 and 2), respectively, which differ from each other in a single nucleotide; and the two probes S and Y, having ~~SEQ ID NO: 12 and 13~~ (SEQ ID NOS:3 and 4), respectively.

At page 63, please replace the paragraph beginning on line 9 with the following amended paragraph.

Two barcode oligo mixes were prepared (one for each barcode oligo) by mixing 20 ~~ul~~ µL of 5x Tth ligase buffer, 15 ~~ul~~ µL of barcode oligonucleotide S or Y at 10 ~~pmoles/ul~~ pmoles/µL; and 62.5 ~~ul~~ µL of water, and 19.5 ~~ul~~ µL of this mix was added to 8 strip tubes. To each strip tube, 0.5 ~~ul~~ µL of respective PCR template S7 or Y7 at 0.04 ~~ug/ul~~ µg/µL was added. The final barcode and template amount was 30 picomoles and 40 femtomoles per reaction, respectively

At page 63, please replace the paragraph beginning on line 14 with the following amended paragraph.

21.5 ~~ul~~ µL of ligase mix that was prepared by mixing 36 ~~ul~~ µL of 5x Tth ligase buffer and 135 ~~ul~~ µL of water, was added to strip tubes 3 and 6 (reactions without ligase). 3.5 ~~ul~~ µL of Tth ligase (50 ~~u/ul~~ U/µL Marsch Bio.) was added to the remaining ligase mix and 21 ~~ul~~ µL of this mix were added to the remaining tubes. The tubes were heated for 1 minute at 65°C, and 20 ~~ul~~ µL of each tube was added to each of the strip tubes containing the DNAs. The volume of each reaction was 40 ~~ul~~ µL.

At page 63, please replace the paragraph beginning on line 19 with the following amended paragraph:

Biotinylated P1Bar primer is identical to P1 bar primer ~~(SEQ ID NO:1)~~ (SEQ ID NO:5) except that it was synthesized with a 5' biotin.

At page 63, please replace the paragraph beginning on line 21 with the following amended paragraph.

For rolling amplification, an extension mix (RCA mix) was prepared by combining (for 20 reactions) 40 ~~ul~~ µL of 10x vent buffer; 20 ~~ul~~ µL DMSO; 64 ~~ul~~ µL of Vent DNA Polymerase exo- at 2 ~~u/ul~~ U/µL (NEB); 3.6 ~~ul~~ µL of P1bar biotin primer (SEQ ID NO:[[1]]5) at 100 ~~pmol/ul~~ pmol/ µL; 9.3 ~~ul~~ µLof T4 gene 32 protein 1.7 ~~m/ml~~ mg/ml; 4 ~~ul~~ µL of MgSO4 at 100 mM; 40 ~~ul~~ µL of each of the four dNTPs at 4 mM; and 179.2 ~~ul~~ µL of water to obtain a final volume of 360 ~~ul~~ µL. 18 ~~ul~~ µLof RCA mix that was prewarmed for 1 minute at 65°C, was added to 2 ~~ul~~ µLof the above reactions, and incubated for 2.5 minutes at 65°C. This results in having 8 tubes each with Taq and Vent elongated biotin P1bar primer.

At page 63, please replace the paragraph beginning on line 28 with the following amended paragraph.

The biotinylated run-off product was isolated using stock Dynabeads (10 ~~ug/ul~~ µg/ µL). These beads can capture up to 20 pmole of biotinyalated oligo using 10 ~~ul~~ µL of stock. 20 ~~ul~~ µL out of the 40 ~~ul~~ µL were taken from each reaction tube and captured with Dynal beads as follows: the stock beads were first washed thrice with 2M NaCl Buffer (~~use~~ using the same volume of buffer as sample); equal volumes of sample and washed beads were combined to obtain a final 1M NaCl mix; this mix was centrifuged at 43°C for 15 minutes at 1400 rpm; the beads were washed twice with 100 ~~ul~~ µL of 2M NaCl buffer and then, once with 100 ~~ul~~ µL double distilled water (by gentle tapping instead, not by pipetting); the beads were resuspended in 50 ~~ul~~ µL of 50 mM NaOH and incubated at room temperature for 5 minutes; the supernatant (which may be neutralized with 5 ~~ul~~ µL of 0.5M HCl) was removed; and the beads were resuspended in original

sample volume (eg. 20 ~~ul~~ μL) using 1X TE.

At page 64, please replace the paragraph beginning on line 9 with the following amended paragraph.

A PCR mix was prepared by mixing 48 ~~ul~~ μL of 10x Taq Gold buffer; 18 ~~ul~~ μL of dNTPs at 4.0 mM; 3.84 ~~ul~~ μL of P1Bar primer (SEQ ID NO:[[1]]5) at 100 ~~pmol/ul~~ pmol/ μL; 3.84 ~~ul~~ μL of M13 primer (SEQ ID NO:[[3]]16) at 100 ~~pmol/ul~~ pmol/ μL; 48 ~~ul~~ μL of MgCl2 at 25 mM; and 330.7 ~~ul~~ μL of water to obtain a total of 456 ~~ul~~ μL l. 1.0 ~~ul~~ μL of bead slurry reaction was added to 19 ~~ul~~ μL PCR mix; denatured for 5 minutes at 95°C ; and subjected to 30 or 40 cycles of PCR as follows: 20 seconds at 95°C and 1 minute at 60°C.

At page 64, please replace the paragraph beginning on line 14 with the following amended paragraph.

20 ~~ul~~ μL of each reaction was then subjected to electrophoresis in 2% agarose , and the bands were visualized as described in the previous examples. The results indicate that more amplification product was obtained in reactions in which the probe perfectly matches the template DNA and ligase is included, i.e., in reactions 2 and 5. In addition, isolation of the run-off product on beads allows cleaner amplification.

At page 65, please replace the paragraph beginning on line 1 with the following amended paragraph.

Two barcode oligo mixes were prepared (one for each barcode oligo) by mixing 10 ~~ul~~ μL of 5x Tth ligase buffer, 15 ~~ul~~ μL of barcode oligonucleotide S (SEQ ID NO:[[12]]3) or Y (SEQ ID NO:[[13]]4)at 10 ~~pmol/ul~~ pmol/ μL; and 72.5 ~~ul~~ μL of water. 19.5 ~~ul~~ μL of this mix was added to 8 strip tubes. To each strip tube, 0.5 ~~ul~~ μL of respective PCR template S7 or Y7 at 0.40 ~~ug/ul~~ μg/μL was added. The final barcode and template amount was 30 picomoles and 40 femtomoles per reaction, respectively.

At page 65, please replace the paragraph beginning on line 6 with the following amended paragraph.

The reaction mixtures (containing the DNAs) were denatured for 5 minutes at 95 °C and annealed for 15 minutes at 65 °C. 23.75 ~~ul~~ µL of ligase mix prepared by combining 24 ~~ul~~ µL of 10 x ~~pfu~~ Pfu ligase buffer and 204 ~~ul~~ µL of water, were added into strip tubes 3 and 6. 10 ~~ul~~ µL of ~~pfu~~ Pfu ligase at 4 ~~u/ul~~ U/µL (Stratagene) was added to the remaining mix of 204.25 ~~ul~~ µL. To each tube (except tubes 3 and 6), 20 ~~ul~~ µL of ligase mix prewarmed for 1 minute at 65 °C was added, and the reactions were incubated for 10 minutes at 65 °C (ligation reactions). The final reaction volume was 40 ~~ul~~ µL.

At page 65, please replace the paragraph beginning on line 12 with the following amended paragraph.

2 ~~ul~~ µL of ligation reactions were added to 18 ~~ul~~ µL of extension mix, which was prepared by combining 40 ~~ul~~ µL of 10 x Taq Gold buffer; 15 ~~ul~~ µL of dNTPs at 4 mM each; 3 ~~ul~~ µL of AmpliTaq Gold DNA Polymerase at 5 ~~u/ul~~ U/µL (P.E.); 3.2 ul biotin RCAP1Bar primer (5' GTCGTTTTACAGACTAGAGACCTCGTGGAC 3' (SEQ ID NO:[[28]]17) at 100 ~~pmol/ul~~ pmol/ µL (same as in example 3); 40 ~~ul~~ µL of MgCl2 at 25 mM; and 258.8 ~~ul~~ µL of water to obtain a final volume of 360 ~~ul~~ µL of PCR reaction mix. The reactions were then incubated for 10 minutes at 95°C to denature the ligated product as well as to activate Taq Gold. ~~One set of reactions was then incubated for 2 minutes at 65 °C, and another set of reactions was The~~One set of reactions was then incubated for 15 minutes at 65 °C to run-off and another set of reactions was not incubated at 65 °C (no run-off control). This resulted in 2 x 8 tubes with Taq elongated biotin RCA primer. The RCA biotin primer contains sequence appended to the 5' end of the P1 primer and was used to increase the distance between the priming sequences and the bead in case the bead sterically hindered the PCR reaction.

At page 65, please replace the paragraph beginning on line 24 with the following amended paragraph.

Two PCR mixes were prepared as described in Example 3 with and without the addition of 1 ~~ul~~ μL per reaction of uracil-N-glycosylase (PE Biosystems, Foster City, CA). 1.0 ~~ul~~ μL of extension reaction was added to 19 ~~ul~~ μL PCR mix; denatured for 5 minutes at 95 °C; and subjected to 25 cycles of PCR as follows: 20 seconds at 95 °C and 1 minute at 64 °C. Also, as a control, 1 ~~ul~~ μL of a 1:10 dilution of the ligation reaction (no extension) was added to 19 ~~ul~~ μL PCR mix, denatured for 5 minutes at 95 °C; and subjected to 25 cycles of PCR as follows: 20 seconds at 95 °C and 1 minute at 64 °C.

At page 65, please replace the paragraph beginning on line 30 with the following amended paragraph.

20 ~~ul~~ μL of each reaction was then subjected to electrophoresis in 2% agarose , and the bands were visualized as described in the previous examples. The results indicate that, in the no extension controls, all background is eliminated by UNG digestion of the probe (~~lanes~~ reactions 1,3,4,6,7,8). In addition, this control shows that the specific signal (~~lanes~~ reactions 2 and 5) are also eliminated without the extension step, thus confirming that the original probe is degraded by UNG and that extension is required for signal. The ~~extendedsion~~ extension experiments indicate that UNG eliminates the background (~~lanes~~ reactions 1, 3 ,4, 6, 7, 8) but not the specific signal (~~lanes~~ reactions 2 and 5).

At page 66, please replace the paragraph beginning on line 25 with the following amended paragraph.

Three template/barcode mixes were prepared by mixing in each 6 ~~ul~~ μL of 10 x ~~pfu~~ Pfu ampligase buffer; 1.8 ~~ul~~ μL of barcode oligo (having the sequence set forth in SEQ ID NO:[[19]]12); 3 ~~ul~~ μL of PCR template (either S7 SEQ ID NO:[[10]]1, Y7 SEQ ID NO:[[11]]2, or water; these templates are the same as those used in Example 1); and

49.2 ~~ul~~ µL of water to obtain a final volume of 60 ~~ul~~ µL. 12 ~~ul~~ µL of each were distributed into tubes.

At page 66, please replace the paragraph beginning on line 29 with the following amended paragraph.

12 ~~ul~~ µL of ligase mix was aliquoted into 16 strip tubes. The mix was prepared for the various reactions as described in Table 5, and the ligase dilution was prepared by mixing 5 ~~ul~~ µL of 10 x ampligase buffer with 44.33 ~~ul~~ µL of water and 0.67 ul of Ampligase at 5 ~~u/ul~~ U/µL, resulting in a solution containing 0.067 ~~u/ul~~ U/µL of Ampligase.

At page 67, please delete Table 5 and replace it with the following Table 5.

Table 5: Preparation of ligase mixes

Ligase mix	each	Rxn 1-8, 13, 16 (x 16)	Rxn 9 -12 (x 8)	Rxn 14 and 15 (x 4)
10x ampligase buffer	1.0 ~~ul~~ µL	16.0 ~~ul~~ µL	8.0 ~~ul~~ µL	4.0 ~~ul~~ µL
Ampligase dilution	0.125 µL	2.0 ~~ul~~ µL	1.0 ~~ul~~ µL	N/A
Taq DNA Pol. Stoffel frag 10 ~~u/ul~~ U/µL	0.05 ~~ul~~ µL	0.8 ~~ul~~ µLl	0.4 ~~ul~~ µL	N/A
Apyrase 50 ~~mU/ul~~ mU/µL	0.2 ~~ul~~ µL l	3.2 ~~ul~~ µL	N/A	0.8 ~~ul~~ µL
H2O		106 ~~ul~~ µL	54.6 ~~ul~~ µL	27.2 ~~ul~~ µL
Total	8.0 ~~ul~~ µL	128.0 ~~ul~~ µL	64.0 ~~ul~~ µL	32.0 ~~ul~~ µL

At page 67, please replace the paragraph beginning on line 12 with the following amended paragraph.

The barcode/~~tempate~~ template mixes were denatured for 5 minutes at 95 °C and annealed for 15 minutes at 65 °C. 8 ~~ul~~ µL ligase mixes were added to the annealed

DNA mixes. These were then incubated for 2 min at 20 °C degrees. The barcode/tempate mixes were then denatured for 5 minutes at 95 °C and annealed for 15 minutes at 65 °C. The temperature was raised to 65 °C and 2 ~~ul~~ µL dXTP (1mM) were added to the appropriate tubes, following which they were incubated for 10 min at 65 °C. Final reaction volume was 20 ~~ul~~ µL. Final enzyme ligase concentration was .00042 ~~units/ul~~ units/µL in the ligation reaction (.0084 units total), the final barcode concentration was 0.015 ~~pmol/ul~~ pmol/ µL and the final template concentration was approximately 2 ~~femtomoles/ul~~ femtomole/µL. ~~[Please confirm or infirm this sequence of steps]~~

At page 67, please replace the paragraph beginning on line 20 with the following amended paragraph.

2 ~~ul~~ µL of each ligation reaction were added to 18 ~~ul~~ µL of PCR extension mix, prepared by combining 85 ~~ul~~ µL of 4x E/U buffer (4x Taq Gold buffer; 3.2 picomoles per microliter P1 bar primer (SEQ ID NO:[[1]]5); 10 mM MgCl2; 0.6 mM dNTPs); 2.55 ~~ul~~ µL of AmpliTaq Gold DNA Polymerase (P.E. Biosystems, Foster City, CA) at 5 ~~u/ul~~ U/µL and 218.5 ~~ul~~ µL of water to obtain a final volume of 306 ~~ul~~ µL. The reactions were incubated for 10 minutes at 95°C to denature the ligated product as well as to activate Taq Gold. The reactions were then incubated for 2 minutes at 65°C to run-off.

At page 67, please replace the paragraph beginning on line 26 with the following amended paragraph.

UNG clean up and amplification were conducted as follows. To each reaction (20 ~~ul~~ µL), 20 ~~ul~~ µL of UNG/PCR mix was added. This mix was prepared by combining 85 ~~ul~~ µL of ~~4 x~~ 4x E/U buffer; 2.55 ~~ul~~ µL of AmpliTaq Gold DNA Polymerase (P.E.) at 5 ~~u/ul~~ U/µL; 17 ~~ul~~ µL of UNG (~~1unit/ul~~ 1unit/ µL PE Biosystems, Foster City, CA); 5.44 ~~ul~~ µL of M13 primer (SEQ ID NO:[[3]]16) at 100 ~~pmol/ul~~ pmol/ µL and 230 ~~ul~~ µL of water to obtain a final volume of 340 ~~ul~~ µL. The reactions were incubated for 20

minutes at 37 °C and then heat denatured for 5 minutes at 95 °C. PCR was conducted for 33 cycles as follows: 20 seconds at 95 °C and 1 minute at 60 °C.

Please replace the paragraph beginning at line 11 of page 68 with the following amended paragraph:

The combinations of template and probe are were as shown in Table 6. The DNA templates were 600 bp DNA fragments amplified from S. cerevisiae. The template S7 ~~(SEQ ID NO: 10~~ (SEQ ID NO:1) is described in Example 1. Template S37 is a 600 bp long double stranded DNA amplified from S. cerevisiae strain S288C, which includes the nucleotide sequence 5' CCAGTCCCTTGAGTTCGCGAATAGTAATTTTGGTGATACCTG 3'~~(SEQ ID NO: 179)~~ (SEQ ID NO:18).:.. The barcode oligonucleotides are SNP2 ~~(SEQ ID NO: 19)~~ (SEQ ID NO:12) and SNP5 ~~(SEQ ID NO: 21)~~ (SEQ ID NO:14).

At page 68, please replace the paragraph beginning on line 22 with the following amended paragraph.

DNA template/probe reaction mixtures were prepared as set forth in Table 7. The enzyme mix listed in the table was prepared by mixing 154.3 ~~ul~~ µL water; 22 ~~ul~~ µL of ~~10 x~~ 10x ampligase buffer; 2.2 ~~ul~~ µL of Apyrase at 50 ~~mU/ul~~ mU/ µL; 1.38 ~~ul~~ µL of Ampligase dilution (5 ~~ul~~ µL of 10 x ampligase buffer; 44.33 ~~ul~~ µL of water and 0.67 ~~ul~~ µLof Ampligase at 5 ~~u/ul~~ U/µL); and 0.55 ~~ul~~ µLof Taq DNA Pol. Stoffel fragment at 10 ~~u/ul~~ U/µL.

At page 68, please delete Table 7 and replace it with the following Table 7.

Table 7: Components of DNA/enzyme mix

DNA/Enzyme mix	Mix 1&2 (x2.5)	Mix 3&4 (x2.5)	Mix 5-8 (x5)
Enzyme mix	41.0 ~~ul~~ µL	41.0 ~~ul~~ µL	82.0 ~~ul~~ µL
Template S-7	1.25 ~~ul~~ µL (S7)		2.5 ~~ul~~ µL (S7)
Template S-37		1.25 ~~ul~~ µL	2.5 ~~ul~~ µL

		(S37)	(S37)
SNP2 1 ~~pmol/ul~~ pmol/μL	0.75 ~~ul~~ μL (SNP2)		1.5 ~~ul~~ μL (SNP2)
SNP5 1 ~~pmol/ul~~ pmol/μL		0.75 ~~ul~~ μL (SNP5)	1.5 ~~ul~~ μL (SNP5)
Total	45.0 ~~ul~~ μL	45.0 ~~ul~~ μL	90.0 ~~ul~~ μL

Please replace the paragraph beginning on page 68, line 35 and ending on page 69, line 6 with the following amended paragraph.

18 ~~ul~~ μL of the mix were distributed into strip tubes. The ~~potential~~ potentially contaminating nucleotides ~~dXTPs~~ were degraded by incubation of the reactions for 4 minutes at 20°C. The reactions were then heated for 5 minutes at 95°C and annealed by incubation for 15 minutes at 65°C. 2 ~~ul~~ μL of the respective dXTPs at 0.1 mM, set forth in Table 6, were added to the reactions and the reactions were incubated for 10 minutes at 65 °C (ligation reactions). In the ligation reaction (20 ~~ul~~ μL), the final barcode concentration was 0.015 ~~picomoles/ul~~ picomoles/μL and template was approximately 2 ~~femtomoles/ul~~ femtomoles/μL. Final ligase concentration was .00042 ~~units/ul~~ units/μL in the ligation reaction (.0084 units total).

At page 69, please replace the paragraph beginning on line 7 with the following amended paragraph.

6 ~~ul~~ μL of ligation reactions were added to 54 ~~ul~~ μL of extension mix prewarmed for 1 minute at 95°C. The extension mix was prepared by combining 54~~ul~~ μL of 10 x Taq Gold buffer; 4.05 ~~ul~~ μL AmpliTaq Gold DNA Polymerase at 5 ~~u/ul~~ u/ μL; 64.8 ~~ul~~ μL of dNTPs at 1.25mM each; 54 ~~ul~~ μLof ~~MgCl2~~ $MgCl_2$ at 25 mM; 4.32 ~~ul~~ μL of P1~~BAR~~ bar (SEQ ID NO[[1]]:5) biotin primer at 100 ~~pmol/ul~~ pmol/μL; and 101.61 ~~ul~~ μL of water.

At page 69, please replace the paragraph beginning on line 14 with the following amended paragraph.

Three cleanups were performed: UNG cleanup, a low stringency biotin cleanup (3 washes), and an increased stringency biotin cleanup (6 washes). 20~~ul~~ μL of each reaction were subjected to capture on Dynal beads. The stock beads were washed thrice with 2M NaCl Buffer using the same volume of buffer as that of the sample. To 25 ~~ul~~ μL of beads were added 75 ~~ul~~ μL 1M NaCl. 20 ~~ul~~ μL of sample were mixed with 80~~ul~~ μL of beads in NaCl to get final 1M NaCl mix and incubated at 43°C for 15 min, pipetting up and down every 5 minutes. The beads were then washed 3 or 6 times in 200 ~~ul~~ μL of 0.5 M NaCl / 0.5 M NaOH buffer, followed by a wash with 200~~ul~~ μL of 0.5 M NaCl in TE. The beads were resuspended in 200 ~~ul~~ μL of: 100 mM NaCl, TE, 0.25% DMSO, 0.01% Triton, and heated for 15-20 min at 70°C. This releases non-specifically bound product to beads. The beads were then washed again with 200 ~~ul~~ μL TE. The beads were resuspended in original sample volume (eg. 20 ul) using 1X TE.

At page 69, please replace the paragraph beginning on line 24 with the following amended paragraph.

Amplification of the cleaned up extension product was carried out by mixing 20 ~~ul~~ μL of the extension product with 20 ~~ul~~ μL of UNG/PCR mix prepared by combining 18 ~~ul~~ μL 10 x Taq~~AQ~~ Gold buffer; 1.35 ~~ul~~ μL AmpliTaq gold DNA polymerase at ~~u/ul~~ 5 U/μL; 21.6 ~~ul~~ μL of dNTPs at 1.25 mM each; 18 ~~ul~~ μL of MgCl2 at 25 mM; 1.44 ~~ul~~ μL P1Bar primer (SEQ ID NO:[[1!]]5) at 100 ~~pmol/ul~~ pmol/μL; 1.44 ~~ul~~ μL M13 primer (SEQ ID NO:[[3]]16) at 100 ~~pmol/ul~~ pmol/μL; 9 ~~ul~~ μL of UNG at 1 ~~unit/ul~~ unit/ μL; and 109.17 ~~ul~~ μL of water. The reactions were incubated for 20 minutes at 37 °C, heat denatured for 5 minutes at 95 ° C and subjected to 14 PCR cycles including 20 seconds denaturation at 95°C; 1 minute annealing at 63 °C; and 10 seconds extension at 72 °C; followed by 20 cycles of 20 seconds at 95 °C; 45 seconds at 56 °C and 10 seconds at 72°C. The reactions were incubated for another 10 seconds at 72 °C and then at 4 °C.

At page 70, please replace the paragraph beginning on line 1 with the following amended paragraph.

The reaction products were analyzed in the same way as in the previous example. The results show that, as expected, a stronger amplification signal was obtained in ~~lanes~~ reactions 2, 3, 6 and 7 (which correspond to reactions including dNTPs that are complementary to the SNP in the template DNA) relative to the other ~~lanes~~ reactions. Since ~~lanes~~ reactions 6 and 7 comprise the two template DNAs and the same two probes and that the reactions were identical except for addition of dCTP in one reaction and dGTP in the other reaction, these results show that two different SNPs can be identified ~~using~~ in the same reaction if the two dNTPs are included in the same ~~reaction.s~~ reactions.

At page 70, please replace the paragraph beginning on line 13 with the following amended paragraph.

Accordingly, 1 ~~ul~~ μL of DraI enzyme was added to 20 ~~ul.~~ μL of PCR product of reactions 6 and 7 and incubated at 37° C for 1 hour. The results show that, as expected, the amplification product observed in reaction 6 corresponds to probe SNP2, whereas that observed in reaction 7 corresponds to probe SNP7. These results provide further support for multiplexing.

Please replace the paragraph beginning at line 22 of page 70 with the following amended paragraph:

The template/probe combinations are set forth in Table 8. The Ttemplate S37 and the probe SNP5 ~~(SEQ ID NO: 21)~~ (SEQ ID NO:14) were was described in the previous Example. SNP5 was described in Example 2 ~~(SEQ ID NO: 21)~~ (SEQ ID NO:14). SNP5 2 part probe was constructed by ligating part A, comprising the template homology region 1 and primer 1 homology region with part Bcomprising primer 2 homology region, barcode sequence, DraI and template homology region 2. The two

parts were enzymatically ligated with a bridging oligonucleotide having the sequence 5' ACTGGCCGTCGTTTTACA/GACTAGAGACCTCGTGGAC 3' (~~SEQ ID NO: 226~~ SEQ ID NO:19; the "/" indicates the portions that are complementary to part A and part B, respectively. Ligation was carried out as follows: 10 picomoles each of SNP5 partA, SNP5 partB, and the bridging oligonucleotide were incubated with 5 units of ampligase, in 1x ampligase buffer for one hour at 60 degrees C The probes contain an uracil base between the primer 2 homology region and the barcode sequence.

At page 71, please replace the paragraph beginning on line 5 with the following amended paragraph.

An enzyme mix was prepared by combining 148.3 ~~ul~~ µL of water, 20 ~~ul~~ µL of ~~pfu~~ Pfu ampligase buffer; 5 ~~ul~~ µL of template S37 at 0.04.~~ug/ul~~ µg/ µL; 2 ~~ul~~ µL of Apyrase at 50 ~~mU/ul~~ mU/µL; 1.25 ~~ul~~ µL Ampligase dilution (5 ~~ul~~ µL 10 x ampligase buffer; 44.33 ~~ul~~ µL water; and 0.67 ~~ul~~ µL Ampligase at 5 ~~u/ul~~ U/µL); and 0.5 ~~ul~~ µL Taq DNA Polymerase Stoffel fragment at 10 ~~u/ul~~ U/µL. DNA enzyme mixes were prepared by combining 79.7 ~~ul~~ µL of enzyme mix with 1.35 ~~ul~~ µL of either probe at 1 ~~pmol/ul~~ pmol/µL. In the ligation reaction (20 ~~ul~~ µL), the final barcode concentration was 0.015 ~~picomoles/ul~~ picomoles/µL, template is approximately 2 ~~femtomoles/ul~~ femtomoles/ µL. Final ligase concentration was 0.00042 ~~units/ul~~ units/ µL in the ligation reaction (.0084 units total).

At page 71, please replace the paragraph beginning on line 12 with the following amended paragraph.

18 ~~ul~~ µL were aliquoted into strip tubes. The ~~dXTPsPotential~~ potentially contaminating nucleotides were degraded by incubation for 4 minutes at 20 °C. The DNA is then denatured by incubation for 5 minutes at 95 °C, and annealed by incubation for 15 minutes at 65 °C. 2 ~~ul~~ µL of respective dXTPs at ~~...~~ 0.1 mM ~~...~~ was added to the appropriate reactions and incubated for 10 minutes at 65 °C (ligation reactions).

At page 71, please replace the paragraph beginning on line 16 with the following amended paragraph.

2 ~~ul~~ µL of ligation reactions were added to 18 ~~ul~~ µL of extension mix prewarmed at 95 °C. Extension mix was prepared by combining 45 ~~ul~~ µL 4 x E/U buffer (described in example 5); 1.35 ~~ul~~ µL of AmpliTaq gold DNA Polymerase at 5 ~~u/ul~~ U/µL and 115,65 ~~ul~~ µL of water. The reactions were incubated for 10 minutes at 95 °C to denature the ~~ligantded~~ product as well as to activate Taq Gold. The reactions were incubated for 2 minutes to runoff, and then brought to 4 °C (extension reaction).

At page 71, please replace the paragraph beginning on line 21 with the following amended paragraph.

UNG cleanup and amplification ~~was~~ were performed by mixing 20 ~~ul~~ µL of extension reaction with 20 ~~ul~~ µL of UNG/PCR mix, prepared by mixing 85 ~~ul~~ µL of 4 x E/U buffer; 2.55 ~~ul~~ µL of AmpliTaq ~~Ggold~~ Gold DNA Polymerase at 5 ~~u/ul~~ U/µL; 17 ~~ul~~ µL UNG at 1 ~~unit/ul~~ unit/ µL; 5.44 ~~ul~~ µL of M13 primer (SEQ ID NO:[[3]]16) at 100 ~~pmol/ul~~ pmol/µL and 230 ~~ul~~ µL of water. The reactions were incubated for 20 minutes at 37 ° C; denatured for 10 minutes at 95 ° C; subjected to 14 PCR cycles of 20 seconds at 95 ° C, 1 minute at 69.6 ° C (decreasing by 0.4 degrees every cycle) and 10 seconds at 72 ° C; followed by 20 PCR cycles of 20 seconds at 95 °C; 45 seconds at 64 °C; and 10 seconds at 72 °C. The reactions were then incubated for 10 seconds at 72 °C and then soaked at 4 °C.

At page 72, please delete the heading on line 1 and replace it with the following heading.

Example 8: Detection of a SNP ~~among~~ in S. cerevisiae genomic DNA

At page 72, please replace the paragraph beginning on line 5 with the following amended paragraph.

PCR Template DNA used in this example was either S. cerevisiae genomic DNA (referred to as genomic template) alone or containing varying concentrations of the template DNA S37 (SEQ ID NO: [[179]] **18), (** described in previous examples). ~~was diluted in S. cerevisiae genomic DNA (referred to as genomic template). To obtain the different diultions of S37 genomic DNA, tThe yeast~~ The probe used in this example was SNP5 (SEQ ID NO:[[21]]14). Probe DNA was first diluted to 0.3 ~~pmol/ul~~ pmol/μL, from which 4 aliquots of 19 ~~ul~~ μL were prepared. 1 ~~ul~~ μL of S37 DNA was added to the first tube, mixed, one ~~ul~~ μL of this dilution was added into the next tube and so on so that the PCR template S37 is serially diluted ~~by~~ in the ~~probegenomic~~ genomic DNA. In reactions 7 and 8, no PCR template is added and only genomic DNA template is present.

At page 72, please replace the paragraph beginning on line 27 with the following amended paragraph.

2 ~~ul~~ μL of each reaction was added to 18 ul of runoff mix prepared by combining (per reaction) 2 ul 10x Taq Gold buffer; 0.75 ~~ul~~ μL dNTPs at 4 mM each; 0.15 ~~ul~~ μL of AmpliTaq ~~gold~~ Gold DNA Polymerase at 5 ~~u/ul~~ U/μL; 0.16 ~~ul~~ μL P1 bar biotin primer (SEQ ID NO[[1]]:5) at 100 ~~pmol/ul~~ pmol/μL; 2 ~~ul~~ μL ~~MgCl2~~ $MgCl_2$ at 25 mM; and 12.94 ~~ul~~ μL water. The reactions were heat denatured for 10 minutes at 95 ° C and runoff products obtained by incubation for 2 minutes at 60 ° C. While the reactions ~~weare~~ were still at 60 ° C, 20 ~~ul~~ μL of the reactions ~~weare~~ were transferred to a UNG/PCR mix prepared by combining 2 ~~ul~~ μL of 10x Taq Gold buffer; 0.75 ~~ul~~ μL dNTPs at 1. ~~ul~~ μL 25 mM each; 0.3 ~~ul~~ μL AmplTaq Gold DNA Polymerase at 100 ~~pmol/ul~~ pmol/μL; 1 ~~ul~~ μL UNG; 0.32 ~~ul~~ μL M13 primer (SEQ ID NO:[[3]]16) at 100 ~~pmol/ul~~ pmol/μL; 2 ~~ul~~ μL ~~MgCl2~~ $MgCl_2$ at 25 mM; and 13.31 ~~ul~~ μL water. The reactions were incubated for 20 minutes at 37 ° C, heat denatured for 5 minutes at 95 ° C and subjected to 14 and 30 amplification cycles of 20 seconds at 95 ° C and 1 minute at 60 ° C each.

At page 73, please replace the paragraph beginning on line 4 with the following amended paragraph.

The amplification products were analyzed as described above. The results show the presence of an amplified product in each ~~lane~~ reaction containing a reaction with a dCTP (the nucleotide complementary to the SNP in the template DNA), but not in ~~lanes~~ reactions containing a reaction with a dGTP. Thus, identification of the SNP was clear even in template DNA highly diluted with yeast DNA. In addition, a strong band was also seen in ~~lane~~ reaction 7, which contained only genomic template and no S37 template, but not in ~~lane~~ reaction 8, which contained dGTP. Thus, this example clearly shows that a SNP can be identified in a unique sequence in genomic DNA.

At page 73, please replace the paragraph beginning on line 11 with the following amended paragraph.

In ~~lanes~~ reactions 7 and 8, with no added PCR template, the only template present is genomic template demonstrating that a SNP can be detected from genomic DNA.

Please replace the paragraph beginning at line 17 of page 73 with the following amended paragraph:

The template DNAs were a mix of 600 base pair PCR templates amplified from S cerevisiae; S-7 ,~~(SEQ ID NO: 10)~~ (SEQ ID NO:1), 26 containing the sequence 5' ACATTTAGATCTGCAGTTTCTAATATGAATTCAGTGGAAAAT 3'~~(SEQ ID NO: 238)~~ (SEQ ID NO:20), 30 containing the sequence 5' GATCAAATGCGACCATATTCATCAAACTTATAGGCG 3' (~~SEQ ID NO: 167 and 37~~ SEQ ID NO:21 containing both sequences 5' TACTGTACCCATTTTTTTGTCGCTTAAGGTTTCGCGT 3' ~~(SEQ ID NO: 5)~~ (SEQ ID NO:22) and ~~SEQ ID NO: 17~~ SEQ ID NO:10) (S37)9.. The probes used were SNPs1, 2, 3, and 5 described previously, e.g., in Example 2. SNP4 (Y4:PL:C:119:159) has the nucleotide sequence

5'ACAAAAAAATGGGTACAGTATAA/UGTCCACGAGGTCTCTAGTC/ /TGTAAAACGACGGCCAGT/UGGTAGTACGGTGCTCTTACA/TTTAAA/ACGCG AAACCTTAAG 3' (SEQ ID NO: 23; representing homology 1/primer1/primer 2/barcode/DraI/homology2; U is uracil). The different combinations of tempate DNA and probes is set forth in Table 10.

At page 74, please replace the paragraph beginning on line 33 with the following amended paragraph.

The reactions were carried out as described in Example 8. Briefly, the template and probe DNAs are combined and incubated with Apryase, Ampligase and Taq DNA Polymerase Stoffel fragment for 4 minutes at 20 °C to degrade the dXTPs. The ~~Eenzyme~~ enzyme mix was prepared by combining 109.1 ~~ul~~ µL of water, 18 ~~ul~~ µL 10 x ~~pfu~~ Pfu Ampligase buffer; 2.7 ~~ul~~ µL of each barcode ~~olio~~ oligo; 4.5 ~~ul~~ µL of each template DNA; 1.8 ul Apyrase at 50m ~~U/ul~~ U/ µL; 1.125 ~~ul~~ µL Ampligase dilution (5~~ul~~ µL Ampligase buffer; 44.33 µL water and 0.67 ~~ul~~ µL Ampligase 5 ~~u/ul~~ U/µL); and 0.45 ~~ul~~ µL Taq DNA Polymerase Stoffel fragment at 10 ~~u/ul~~ U/µL. 18 ~~ul~~ µL of the mix were transferred to strip tubes, which were incubated for 4 minutes at 20 °C to degrade potential contaminating nucleotides. The reactions were then denatured by incubation at 95 °C for 5 minutes and annealed at 65 °C for 15 minutes. 2~~ul~~ µL of the respective dXTP was added and the reactions incubated for 10 minutes at 65 °C. In the ligation reaction (20 ~~ul~~ µL), the final barcode probe concentration ~~wais~~ was 0.015 ~~picomoles/ul~~ picomoles/µL and, template concentration wais approximately 2 ~~femtomoles/ul~~ femtomoles/µL. Final ligase concentration ~~iwas~~ was 0.00042 ~~units/ul~~ units/ µL in the ligation reaction (.0084 units total).

At page 74, please replace the paragraph beginning on line 46 with the following amended paragraph.

2 ~~ul~~ µL of each reaction was added to 18 ~~ul~~ µL of runoff mix preheated to 95 ° C

prepared by combining 34 ~~ul~~ µL 10x Taq Gold buffer; 40.8 ~~ul~~ µL dNTPs at 1.25 mM each; 2.25 ~~ul~~ µL of AmpliTaq gold DNA Polymerase at 5 ~~u/ul~~ U/µL; 2.72 ~~ul~~ µL P1bar biotin primer (SEQ ID NO:[[1]]5) at 100 ~~pmol/ul~~ pmol/µL; 34 ~~ul~~ µL MgCl2 at 25 mM; and 306 ~~ul~~ µL water. The reactions were heat denatured for 10 minutes at 95 ° C and runoff products obtained by incubation for 2 minutes at 60 ° C. The reactions were then brought to 4 ° C .

At page 74, please replace the paragraph beginning on line 51 with the following amended paragraph.

Biotin cleanup was performed as described in Example 6. Briefly, the beads were washed as described and resuspended in 2 volumes 2M NaCl. 20 ~~ul~~ µL of each reaction were added to 20 ~~ul~~ µL of beads to get a 1M NaCl mix. The mix was incubated at 43 °C for 15 min, pipetting up and down every 5 minutes. The beads were then washed 6 times in 200 ~~ul~~ µL of 0.5 M NaCl / 0.5 M NaOH buffer, followed by a wash with 200 ~~ul~~ µL of 0.5 M NaCl in TE. The beads were resuspended in 200 ~~ul~~ µL of: 100 mM NaCl, TE, 0.25% DMSO, 0.01% Triton, and heated for 15-20 min at 70 °C. This releases non-specifically bound product to beads. The beads were then washed again with 200 ~~ul~~ µL TE. The beads were resuspended in original sample volume (eg. 20 ~~ul~~ µL) using 1X TE.

At page 74, please replace the paragraph beginning on line 59 with the following amended paragraph.

20 ~~ul~~ µL of the reactions were transferred to a UNG/PCR mix prepared by combining 18 ~~ul~~ µL of 10x Taq Gold buffer; 21.6 ~~ul~~ µL dNTPs at 1.25 mM each; 1.35 ~~ul~~ µL ~~AmplTaq~~ AmpliTaq Gold DNA Polymerase at 100 ~~pmol/ul~~ pmol/µL; 1.44 ~~ul~~ µL P1 ~~Bar~~ bar primer (SEQ ID NO:[[1]]:5) at 100 ~~pmol/ul~~ pmol/µL; 9 ~~ul~~ µL UNG; 2.88 ~~ul~~ µL M13 biotin primer (SEQ ID NO:[[3]]16) at 100 ~~pmol/ul~~ pmol/µL; 18 ~~ul~~ µL ~~MgCl2~~ $MgCl_2$ at 25 mM; and 107.9 ~~ul~~ µL water. The reactions were incubated for 20 minutes

at 37 ° C, heat denatured for 5 minutes at 95 ° C and subjected to 14 amplification cycles of 20 seconds at 95 ° C; 1 minute at 69.6 ° C (decreasing by 0.4 ° C every cycle); and 10 seconds at 72 ° C and 20 amplification cycles of 45 seconds at 64 ° C; and 10 seconds at 72 ° C. The reactions are then incubated for 10 seconds at 72 ° C and further incubated at 4 ° C.

At page 75, please replace the paragraph beginning on line 10 with the following amended paragraph.

The amplified products were ~~furtherthen~~ further analyzed by hybridization of each multiplexed reaction to a DNA chip. Each dXTP reaction (multiplexed to 5 probes) was hybridized to a separate chip. In each case, the hybridization mixture consisted of the following: 2.0 ~~ul~~ μL of the above PCR reaction, 0.5 ~~ul~~ μL of a control (border) oligo at 0.7 ~~fm/ul~~ fm/μL, 2.9 ~~ul~~ μL M13 complement oligo at 10 ~~pm/ul~~ pm/μL (10-fold excess over the M13 primer of the PCR reaction), brought up to 160 ~~ul~~ μL in 6X SSPE-T buffer (6X SSPE buffer with 0.005% Triton). This mixture was denatured for 2 min at 95 ° C ~~C~~ and then ~~put~~ incubated on ice for 5 min. The solution was loaded on a DNA chip and hybridized at 42 ° C ~~C~~ for 4 hours. After this period, the chip was washed with 6X SSPE-T, 5 times and loaded with the following for fluorescent labeling: 0.5 ~~ul~~ μL of Streptavidin R-Phycoerythrin conjugate (1 mg/ml), 10 ~~ul~~ μL of BSA (20 mg/ml), brought up to 160 ~~ul~~ μL in SSPE-T buffer. The chip was incubated for 10 minutes at 42 C. After this, the chip was again washed with SSPE-T buffer 5 times and loaded onto a laser fluorescence scanner for analysis of the multiplexed reaction products. The signal at each of the five probe features of interest were averaged over the 8x8 pixels per feature, background subtracted and then normalized using the average signal intensity of the control (border) features. This effectively normalized the difference in hybridization efficiency on the four different chips. Table 11 shows normalized signal intensity from four hybridizations, one for each nucleotide. The signal : noise ratio corresponds to the normalized signal at the expected nucleotide to

the highest normalized signal at the other three nucleotides

At page 76, please replace the paragraph beginning on line 8 with the following amended paragraph.

The template DNA from S. cerevisiae (S96 genomic DNA at 197 ~~ng/ul~~ ng/μL ~~[what is S96 DNA?we tested two strain of yeast S96 and YJM, in all examples, S96 was used]~~) was incubated with one or more SNP probes, as set forth in Table 12. The sequences of the two part probes are provided in the previous examples.

At page 76, please replace the paragraph beginning on line 22 with the following amended paragraph.

The reactions were carried out as described in Example 9. Briefly, the template and probe DNAs were combined and incubated with Apryase, Ampligase and Taq DNA Polymerase Stoffel fragment for 4 minutes at 20 °C to degrade the dXTPs. An enzyme mix was prepared by combining 409.95 ~~ul~~ μL of water, 60 ~~ul~~ μL 10 x ~~pfu~~ Pfu Ampligase buffer; 15.3 ~~ul~~ μL of template DNA at 197 ~~ng/ul~~ ng/μL; 6 ~~ul~~ μL Apyrase at 50 ~~mU/ul~~ mU/μL; 0.75 ~~ul~~ μL Ampligase; and 3 ~~ul~~ μL Taq DNA Polymerase Stoffel fragment at 10 ~~u/ul~~ U/μL. 18 ~~ul~~ μL were transferred to strip tubes. The final mix was prepared by combining (for 5 reactions) 74.25 ~~ul~~ μL enzyme mix; 1.35 ~~ul~~ μL of each barcode oligoprobe and TE if necessary to obtain a volume of 81 ~~ul~~ μL.

Please replace the paragraph beginning on page 76, line 29 and ending on page 77, line 1, with the following amended paragraph.

The reactions were then denatured by incubation at 95 °C and annealed at 65 °C for 15 minutes. 2 ~~ul~~ μL of the respective dXTP at 0.1 mM was added and the reactions were incubated for 10 minutes at 65 °C. In the ligation reaction (20 ~~ul~~ μL), the final barcode probe concentration ~~wais~~ was 0.015 ~~picomoles/ul~~ picomoles/μL.

At page 77, please replace the paragraph beginning on line 2 with the following amended paragraph.

3 ~~ul~~ μL of each reaction was added to 27 ~~ul~~ μL of runoff mix prepared by combining 78 ~~ul~~ μL 10x Taq Gold buffer; 93.6 ~~ul~~ μL dNTPs at 1.25 mM each; 5.85 ~~ul~~ μL of AmpliTaq ~~gold~~ Gold DNA Polymerase at 5 ~~u/ul~~ U/μL; 6.24 ~~ul~~ μL P1bar biotin primer (SEQ ID NO[[1]]:5) at 100 ~~pmol/ul~~ pmol/μL; 78 ~~ul~~ μL ~~MgCl2~~ $MgCl_2$ at 25 mM; and 440.31 ~~ul~~ μL water. The reactions were heat denatured (and Taq activated) for 10 minutes at 95 °C and runoff products obtained by incubation for 2 minutes at 60 °C. The reactions were then~~, and~~ chilled by incubation at 4 °C .

At page 77, please replace the paragraph beginning on line 8 with the following amended paragraph.

20 ~~ul~~ μL of the reactions were transferred to a UNG/PCR mix prepared by combining 78 ~~ul~~ μL of 10x Taq Gold buffer; 93.6 ~~ul~~ μL dNTPs at 1.25 mM each; 78 ~~ul~~ μL AmpiTaq Gold DNA Polymerase; 39 ~~ul~~ μL UNG; 12.48 ~~ul~~ μL M13 primer (SEQ ID NO:[[3]]16) at 100 ~~pmol/ul~~ pmol/μL; 6.24 ~~ul~~ μL P1~~Bar~~ bar primer (SEQ ID NO:[[1]]5) at 100 ~~pmol/ul~~ pmol/μL; 78 ~~ul~~ μL ~~MgCl2~~ $MgCl_2$ at 25 mM; and 466.83 ~~ul~~ μL water. The reactions were incubated for 20 minutes at 37 °C, heat denatured for 10 minutes at 95 °C and subjected to 14 amplification cycles of 20 seconds at 95 °C; 1 minute at 69.6 °C (decreasing by 0.4 °C every cycle), followed by 30 amplification cycles of 20 seconds at 95 °C; 45 seconds at 64 °C; and 10 seconds at 72 °C. The reactions ~~weare~~ were then incubated for 10 seconds at 72 °C and then soaked at 4 °C.

At page 77, please replace the paragraph beginning on line 16 with the following amended paragraph.

The amplification products were analyzed as described in Example 8. The results clearly show the presence of amplification products in reactions in which the dNTP that was added is complementary to the SNP in the template DNA. For example, ~~lane~~

reaction 7 shows a reaction with a SNP2 probe and dGTP, which is the nucleotide that is complementary to the SNP in the template DNA at that location. ~~Similary,~~ Similarly, ~~lane~~ reaction 18 shows an amplification product resulting from the addition of dCTP which is the complementary nucleotide to SNP5 in template DNA. In reactions 22, 23 and 24, bands are also clearly visible indicating that amplification does occur in multiplexed reactions.

At page 77, please replace the paragraph beginning on line 23 with the following amended paragraph.

The dCTP and the dGTP nucleotide reactions were also analyzed by hybridization to DNA chips. The hybridization conditions were similar to those in the example 9, except that 20 ~~ul~~ μL of the PCR reaction was used in the hybridization mix and the chip was hybrizided for 12 hours. Table 13 shows normalized signal intensity from the two hybridizations. The ~~Signal:Noise~~ signal:noise ratio corresponds to the normalized signal at the expected nucleotide to the normalized signal at the other nucleotide.

At page 78, please replace the paragraph beginning on line 6 with the following amended paragraph.

The reactions are set forth in Table 14[[3]]. Yeast genomic DNA (200 ~~ng/ul~~ ng/μL) was serially diluted into calf thymus (100 ~~ng/ul~~ ng/μL) as follows. 1 ~~ul~~ μL of yeast S96 was mixed with 19 ~~ul~~ μL of calf thymus (Dilution 1). 2 ~~ul~~ μL of Dilution 1 were mixed into 18 ~~ul~~ μL of calf thymus (Dilution2). 2 ~~ul~~ μL of Dilution 2 were mixed into 18 ul of calf thymus (Dilution 3).

At page 78, please replace the single line paragraph at line 10 with the following amended paragraph.

Table [[143]]14: Components of the reactions

At page 78, please replace the paragraph beginning on line 19 with the following amended paragraph.

An enzyme mix containing the template and probe DNAs was prepared by combining (per reaction) 4.875; 11.875; 13.875; or 14.575 ~~ul~~ µL of water; 2 ul 10 x ~~pfu~~ Pfu Ampligase buffer; 0.3 ~~ul~~ µL of barcode ~~olio~~ oligo; 10, 3, 1, or 0.3 ~~ul~~ µL of yeast genomic dilution; 0.2 ~~ul~~ µL Apyrase at 50 ~~mU/ul~~ mU/µL; 0.125 ~~ul~~ µL ~~ul~~ µL Ampligase; and 0.5 ~~ul~~ µL ~~1~~ Taq DNA Polymerase Stoffel fragment at 10 ~~u/ul~~ U/µL. 18 ul were transferred to strip tubes. dXTPs Potential contaminating nucleotides were degraded by incubation for 20 minutes at 4°C. The reactions were then denatured by incubation at 95°C for 5 minutes and ramped down to 65°C. 2 ~~ul~~ µL dXTP at 100uM dilution was added and the reactions were incubated at 65°C for 10 minutes.

At page 79, please replace the paragraph beginning on line 26 with the following amended paragraph.

For Taq run-off, 2 ~~ul~~ µL of ligation mix was added to 18 ~~ul~~ µL of run-off mix and heat denatured for 10 minutes at 95 °C. Runoff mix was prepared by combining (per reaction) 2 ~~ul~~ µL 10x Taq Gold buffer; 0.75 ~~ul~~ µL dNTPs at 4 mM each; 0.15 ~~ul~~ µL of AmpliTaq ~~gold~~ Gold DNA Polymerase at 5 ~~u/ul~~ U/µL; 0.16 ~~ul~~ µL P1bar biotin primer (SEQ ID NO[[1]]:5) at 10 ~~pmol/ul~~ pmol/µL; 2 ~~ul~~ µL ~~MgCl2~~ $MgCl_2$ at 25 mM; 1 ~~ul~~ µL UNG; and 13.78 ~~ul~~ µL water. The reactions were heat denatured (and Taq activated) for 10 minutes at 95°C and runoff products obtained by incubation for 2 minutes at 60°C.

At page 78, please replace the paragraph beginning on line 32 with the following amended paragraph.

After runoff, while the mixture is still at 60 °C, 20 ~~ul~~ µL of the extension reaction were transferred into a UNG/PCR mix, prepared by combining (per reaction) 2 ~~ul~~ µL of 10x Taq Gold buffer; 0.75 ~~ul~~ µL dNTPs at 1.25 mM each; 0.15 ~~ul~~ µL AmpiTaq Gold DNA

Polymerase at 5 ~~units/ul~~ units/μL; 1 ~~ul~~ μL UNG; 0.16 ~~ul~~ μL M13 primer (SEQ ID NO[[3]]:16) at 100 ~~pmol/ul~~ pmol/μL; 0.16 ~~ul~~ μL P1~~Bar~~ bar primer (SEQ ID NO[[1]]:5) at 100 ~~pmol/ul~~ pmol/μL; 2 ~~ul~~ μL ~~1~~ ~~MgCl2~~ $MgCl_2$ at 25 mM; and 13.78 ~~ul~~ μL water. The reactions were incubated for 20 minutes at 37 °C , heat denatured for 5 minutes at 95 °C and subjected to 35 amplification cycles of 20 seconds at 95 °C; 45 seconds at 64 °C; and 10 seconds at 72 °C. The reactions are then incubated for 10 seconds at 72°C and then soaked at 4 °C.

Please replace the paragraph beginning at line 14 of page 79 with the following amended paragraph:

Two DNA samples were obtained from a Northern European donor and an Indian donor. The samples were screened for two markers in the human ATM gene, GenBank accession number HSU82828. This gene contains many polymorphisms including two SNPs: one at base 46611 (intron 17; G to A: 34,107) and the second one at 60136 (Intron 22; T to C: 35107). The probe designed to detect the SNP at base 46611 was prepared by ligating two oligonucleotides using a bridging oligonucleotide as described above, to produce a probe having the nucleotide sequence 5' AGAATAATTGTTTTTATTTCTTTGAAC/UGTCCACGAGGTCTCTAGTC/ TGTAAAACGACGGCCAGT/UATGCGTACCCTCGACTGAG/TTTAAA/TAGAGA AAACACTGTCTGCC 3' ~~(SEQ ID NO: 264)~~ (SEQ ID NO:24), represented as homology1 / primer1/primer2 / barcode / DraI / homology2 ("U" indicates uracil bases). The probe to detect the second SNP was also constructed by ligating two oligonucleotides using a bridging oligonucleotides, to produce a probe having the nucleotide sequence 5' AATAACCTTTCAGTGAGTTTTGAC/UGTCCACGAGGTCTCTAGTC/ TGTAAAACGACGGCCAGT/UACTGTCACCGGAGTCTGAG/TTTAAA/GACATA TTGGAAGTAACTTA 3' ~~(SEQ ID NO: 275)~~ (SEQ ID NO:25).

Please replace the two single line paragraphs beginning on page 79, line 28 with the following two amended paragraphs.

The compositions of the reactions are set forth in ~~Table 145~~ Table 15.

~~Table 154~~ Table 15: Components of the reactions

At page 80, please replace the paragraph beginning on line 12 with the following amended paragraph.

An enzyme mix containing the template and probe DNAs was prepared by combining 232.7 ~~ul~~ µL of water; 40 ~~ul~~ µL 10 x pfu Ampligase buffer; 4 ~~ul~~ µL Apyrase at 50 ~~mU/ul~~ mU/µL ; 2.5 ~~ul~~ µL Ampligase; and 0.5 ~~ul~~ µL Taq DNA Polymerase Stoffel fragment at 10 ~~u/ul~~ U/µL. Four enzyme/DNA mixes were prepared by combining 65.07 ~~ul~~ µL enzyme mix; 13.5 ~~ul~~ µL of template DNA; and 0.54 ~~ul~~ µL probe DNA. 18 ~~ul~~ µL were transferred to strip tubes. ~~dXTPs Potential~~ Potentially contaminating nucleotides were degraded by incubation for 20 minutes at 4 °C. The reactions were then denatured by incubation at 95 °C for 5 minutes and ramped down to 65 °C for about 15 minutes. 2 ~~ul~~ µL dXTP at 100uM dilution was added and the reactions were incubated at 58 °C for 10 minutes.

At page 80, please replace the paragraph beginning on line 20 with the following amended paragraph.

For Taq run-off, 2 ~~ul~~ µL of ligation mix was added to 18 ~~ul~~ µL of run-off mix warmed to 95 °C, prepared by combining 34 ~~ul~~ µL 10x Taq Gold buffer; 12.75 ~~ul~~ µL dNTPs at 1.25 mM each; 2.55 ~~ul~~ µL of AmpliTaq ~~gold~~ Gold DNA Polymerase at 5 ~~u/ul~~ U/µL; 2.72 ~~ul~~ µL P1bar biotin primer (SEQ ID NO:[[1]]5) at 10 ~~pmol/ul~~ pmol/µL; 34 ~~ul~~ µL ~~MgCl2~~ $MgCl_2$ at 25 mM; and 220 ~~ul~~ µL water. The reactions were heat denatured (and Taq activated) for 10 minutes at 95 °C and runoff products obtained by incubation for 2 minutes at 60 °C, and then chilled at 4 °C.

At page 80, please replace the paragraph beginning on line 25 with the following amended paragraph.

20 ~~ul~~ μL of the extension reaction were transferred into a UNG/PCR mix, prepared by combining 34 ~~ul~~ μL of 10x Taq Gold buffer; 12.75 ~~ul~~ μL dNTPs at 1.25 mM each; 2.55 ~~ul~~ μL ~~AmpiTaq~~ AmpliTaq Gold DNA Polymerase at 5 ~~units/ul~~ units/μL; 17 ~~ul~~ μL UNG 1 ~~unit/ul~~ unit/μL; 2.72 ~~ul~~ μL M13 primer (SEQ ID NO[[3]]:16) at 100 ~~pmol/ul~~ pmol/μL; 2.72 ~~ul~~ μL P1Bar primer (SEQ ID NO[[1]]:5) at 100 ~~pmol/ul~~ pmol/μL; 34 ~~ul~~ μL ~~MgCl2~~ $MgCl_2$ at 25 mM; and 234.26 ~~ul~~ μL water. The reactions were incubated for 20 minutes at 37 ° C, heat denatured for 10 minutes at 95 ° C and subjected to 35 amplification cycles of 20 seconds at 95 ° C; 45 seconds at 64 °C; and 10 seconds at 72 °C. The reactions are then incubated for 10 seconds at 72C ° and then soaked at 4 ° C.

Please replace the paragraph beginning on page 80, line 32 and ending on page 81, line 3, with the following amended paragraph.

The amplification products were analyzed by gel electrophoresis as described in previous examples. The results indicate the presence of an amplification product in ~~lanes~~ reactions 3 and 11 for the ATM46611 SNP indicating that both genomic DNAs are homozygous G for this SNP. Amplification products in ~~lane~~ reaction 6 but not 8 for the ~~nNorthern eEuropean~~ Northern European donor indicates that this genomic DNA is homozygous for C for the ATM60136 SNP while the ~~eEast indian Indian~~ East Indian genomic DNA is heterozygous for C and T due to the presence of products in the ~~lane~~ reaction 14 and 16 ~~lanes~~, respectively.

At page 81, please replace the paragraph beginning on line 12 with the following amended paragraph.

The template DNA used was purified human genomic DNA and the probes used have the nucleotide sequence ~~5'~~

A9

5'TATGACCAGAGGTTTCTGACTGTCCACGAGGTCTCTAGTCTGTAAAACGA CGGCCAGTGGGTACATCCAAGCAACCGAGTTTCCTGGCATTATATCATCT 3' (SEQ ID NO:[[X]]26)

A10

5'ACCTGGAAGCCAACTTCGTCCACGAGGTCTCTAGTCTGTAAAACGACGGC CAGTAGCGTACTCTGAATGCCGTCGCCAGAAATTAGTCAAGGAAA 3' (SEQ ID NO:[[X]]27)

~~A9~~ A9U

5'UTATGACCAGAGGTTTCTGACTGTCCACGAGGTCTCTAGTCUTGTAAAAC GACGGCCAGTGGGTACATCCAAGCAACCGAGTTTCCTGGCATTATATCATCT 3' (SEQ ID NO:[[X]]28)

~~A10~~ A10U

5'UCACCTGGAAGCCAACTTCGTCCACGAGGTCTCTAGTCUTGTAAAACGAC GGCCAGTAGCGTACTCTGAATGCCGTCGCCAGAAATTAGTCAAGGAAA 3' (SEQ ID NO:[[X]]29)

At page 81, please replace the paragraph beginning on line 29 with the following amended paragraph.

A single nucleotide gap fill reaction mix was prepared by mixing 48 ~~ul~~ μL of 10x ampligase reaction buffer (Epicentre), 0.6 ~~ul~~ μL apyrase 500 ~~milliunits/ul~~ milliunits/μL (Sigma), 2.4 ~~ul~~ μL Taq polymerase Stoffel fragment 10 ~~units/ul~~ units/μL (ABI), 0.6 ~~ul~~ μL Ampligase enzyme 5 ~~units/ul~~ units/μL (Epicentre), 24 ~~ul~~ μL human genomic DNA 100 ~~ng/ul~~ ng/μL, and 345 ~~ul~~ μL water. 44.75 ~~ul~~ μL of this reaction mix was added to 0.25 ~~ul~~ μL of each probe (1.25 ~~femptomoles/ul~~ femtomoles/μL) 9 ~~ul~~ μL of which was pippetted into each of four positions in a reaction plate, one for each nucleotide.

At page 81, please replace the paragraph beginning on line 34 with the following amended paragraph.

The reaction mixtures (containing the DNAs) were incubate for 4 minutes at 20 ° C, denatured for 5 minutes at 95 ° C and annealed for 15 minutes at 55 ° C. To each tube 1 ~~ul~~ μL of 1.25 micromolar deoxynucleotide (Pharmacia) was added (as indicated in table [[XX]]16) and the reaction was incubated 10 minutes at 55 ° C. At this point, probes have been circularized around the genomic DNA if the correct nucleotide was added. The reaction mixture was then incubated at 95 °C for 2 minutes and then brought to 37 ° °C. To each well, 25 ~~ul~~ μL of uracil – N – glycosylase mix was added consisting of 2.5 ~~ul~~ μL 10x Taq gold buffer (ABI), 1.6 ~~ul~~ μL 25mM MgCl2, water, and 10 ~~ul~~ μL of UNG (if indicated in table [[XX]]16). The reactions were incubated 20 minutes at 37 °C for depyrimidization, then for 10 minutes at 95 °C to break the abasic site.

Please replace the single line paragraph on page 82, line 6, with the following amended paragraph.

Table [[XX]]16: components of the different reactions.

At page 82, please replace the paragraph beginning on line 15 with the following amended paragraph.

Ligated probe products were amplified by adding 25 ~~ul~~ μL of an amplification mix consisting of 2.5 ~~ul~~ μL 10x Taq gold buffer (ABI), 1.6 ~~ul~~ μL 25mM MgCl2, 2.24 ~~ul~~ μL dNTPs at 1.25 mM each, 0.08 ~~ul~~ μL of M13 primer (SEQ ID NO:[[XX]]16) at 197 ~~pmol/ul~~ pmol/μL, 0.09ul of P1Bar primer (SEQ ID NO:[[XX]]5) at 186 ~~pmol/ul~~ pmol/μL, 0.4 ul Amplitaq Gold DNA polymerase 5 ~~units/ul~~ units/μL (ABI), and water, and thermocycling the mixture 20 seconds at 95 °C, 45 seconds at 64 °C, and 10 seconds at 72 °C for 31 cycles

At page 82, please replace the paragraph beginning on line 20 with the following amended paragraph.

20 ~~ul~~ μL of each reaction was then subjected to electrophoresis in 4% agarose, and the bands were visualized as described in the previous examples. The results indicate the signal, which is a band seen migrating at 100 base pairs as compared to the DNA ladder run to the left, is greatly increased in reactions with probes that contain a uracil and were incubated with uracil – N – glycosylase indicating that both the enzyme and its target uracil on the probe are necessary to release the circularized probe from the genomic DNA template and allow efficient amplification.